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DARPA Antibody Technology Program, Phase II: Characterization of an Anti-BclA Antibody Produced by U.S. Naval Research Laboratory

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14. ABSTRACT:
The Defense Advanced Research Projects Agency (DARPA; Arlington, VA) Antibody Technology Program focused on development of technologies that enhance the thermal stability and binding affinity of a given antibody. In this study, the U.S. Army Edgewood Chemical Biological Center (ECBC; now known as U.S. Army Combat Capabilities Development Command Chemical Biological Center; Aberdeen Proving Ground, MD) functioned as an independent testing laboratory to provide technical support on immune reagents and assist in defining the government-supplied antibody–antigen pairs. Project goals were to (1) implement standardized methods for characterizing antibodies developed at ECBC with de novo thermal and binding properties of select reagents for use by DARPA-funded investigators, and (2) use those methods to validate changes in antibody thermal stability and binding affinities achieved by DARPA investigators. The BA21 antibody, which detects BclA of *Bacillus anthracis,* was chosen for this project. This report describes evaluation of BA21 antibodies (supplied by U.S. Naval Research Laboratory; Washington, DC) for affinity and stability enhancements. Results of this study provide standardized parametric data on antibody properties and performance and contribute to the development of decisional analysis tools for selecting antibody-based reagents that optimize field-operation and performance metrics for future detection and diagnostic platforms.

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PREFACE

The work described in this report was supported by the Defense Advanced Research Projects Agency (Arlington, VA), project number BA08DET000/1R3Z11. The work was started in September 2010 and completed in September 2012.

At the time this work was performed, the U.S. Army Combat Capabilities Development Command Chemical Biological Center was known as the U.S. Army Edgewood Chemical Biological Center.

This report has been approved for public release.

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DARPA ANTIBODY TECHNOLOGY PROGRAM, PHASE II: CHARACTERIZATION OF AN ANTI-BclA ANTIBODY PRODUCED BY U.S. NAVAL RESEARCH LABORATORY

1. INTRODUCTION

In an effort to more fully characterize and compare the physical and functional properties of antibody reagents in its repository, the Joint Product Management Office for Biosurveillance (Fort Detrick, MD) has instituted a quality program for standardization of test methods. Production methods for antibodies used in detection devices have drastically changed over time. Animal models provide the most common method for producing polyclonal antibodies; however, the antibodies lack antigen-binding specificity, and the antibody affinity depends on the individual animals. Development of the monoclonal antibody (mAb) allowed for more specificity; however, the use of large numbers of animals is still required. With the advent of hybridoma cell culture production, larger quantities of high-activity antibodies were produced, and existing cell lines could be panned for higher-affinity antibodies. Recombinant methods have greatly increased researchers' ability to produce more-specific antibodies with antigen-binding fragment (Fab), single-chain variable fragment (scFv), and single-domain antibody (sdAb). Many varying recombinant production systems are being used, from basic bacteria, yeasts, and filamentous fungi to insect cell lines and mammalian cells, and including transgenic plants and animals (*1*).

The Defense Advanced Research Projects Agency (DARPA; Arlington, VA) Antibody Technology Program (ATP) focused on the development of technologies that enhance the thermal stability and binding affinity of a given antibody. Increased thermal stability would eliminate the need for cold storage as well as increase the usability of antibodies in harsh conditions, such as those experienced by troops in the field. An added benefit of controlling affinity is the capability to develop multiplex sensors to detect different numbers and varieties of antigens. The U.S. Army Edgewood Chemical Biological Center (ECBC; now known as the U.S. Army Combat Capabilities Development Command Chemical Biological Center; Aberdeen Proving Ground, MD) functioned as an independent testing laboratory for this program. ECBC personnel provided specific technical support on immune reagents as well as assistance in defining the government-supplied antibody–antigen pairs. Standardized methods developed at ECBC for characterizing antibodies were used for validating the changes in antibody thermal stability and binding affinity that had been achieved by the DARPA investigators. Because combinatorial approaches to antibody enhancement are random and may lead to fortuitous improvements in stability or affinity, the strategies for ATP enhancement were required to be directed so that they could be transferable to other antibody molecules.

The primary objectives for the Phase I ATP were to develop and demonstrate strategies that independently modulate antibody stability and affinity in a viral antibody molecule that was provided by the U.S. Government. The Phase II goal was to modify an antibody using the Phase I techniques and thereby produce 2 g of a single protein that yielded a 100-fold increase in the affinity and a 10 °C increase in the melting temperature of a provided protein. The

focus of the work highlighted in this report is the evaluation of antibodies supplied by the DARPA-funded investigator U.S. Naval Research Laboratory (NRL; Washington, DC) for affinity and stability enhancements. The antibody chosen for this project was BA21, which detects BclA, the bacillus collagen-like protein of *Bacilllus anthracis*. The results of this study provide standardized parametric data on antibody properties and performance and contribute to the development of decisional analysis tools for selecting antibody-based reagents that optimize field-operation and performance metrics for future detection and diagnostic platforms.

2. MATERIALS AND METHODS

2.1 Antibody and Antigen

2.1.1 Anti-*B. anthracis* Antibodies

The Critical Reagents Program collection contained a large number of both polyclonal and monoclonal anti-*B. anthracis* antibodies. For this project, the anti-*B. anthracis* BA21 antibody that detects BclA was chosen.

2.1.2 Modification of BA21

NRL personnel modified the BA21 antibody to produce an sdAb against BclA, as described (*2*). It was named MBP-sdAb.

2.1.3 tBclA

The antigen is the bacillus collagen-like protein of anthracis (BclA). This protein is a major structural component of the spore-coat protein from *B. anthracis,* and it has been shown to be highly immunogenic (*2*). We used a truncated form of the protein (tBclA) that was purified by affinity chromatography. This form leads to dimerization in solution but reduces multimerization (*3, 4*).

2.2 Test Methods

The standardized parametric tests that were established during the MS2 scFv antibody DARPA ATP Phase I investigation (*5*) were used in this study.

2.2.1 Antibody Concentration Measurement

The antibody concentrations were determined using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific; Madison, WI). This instrument provided the absorbance of light at 280 nm (A_{280}) for each sample. Extinction coefficients were used in conjunction with the A_{280} values to determine accurate concentrations. The concentration of each sample was determined by dividing the average A_{280} value by the extinction coefficient for each antibody. Each reading required a 2 µL sample, which was placed on the sample pedestal. The instrument was blanked using phosphate-buffered saline (PBS; Sigma-Aldrich; St. Louis, MO), and readings were taken in triplicate. As a positive control and to validate the instrument operation, bovine γ-globulin (BGG; Bio-Rad; Hercules, CA) was also tested.

2.2.2 Molecular Weight and Purity Measurements

An Experion automated electrophoresis system (Bio-Rad) was used to determine the molecular weight and purity of MBP-sdAb and BA21. The microfluidic chip, in conjunction with the Experion reagents, electrophoresis station, and software, is designed to accomplish separation, staining, destaining, detection, and basic data analysis. Sample purity was determined by calculating the percent mass of the separated proteins in a sample. For Experion analysis, each antibody concentration was standardized by dilution in PBS to a final concentration of 1 mg/mL. The control (BGG) and the antibody samples were then processed using the validated procedure specified in the *Experion Pro260 Analysis Kit Instruction Manual* (*6*). Briefly, a Pro260 microfluidic chip was prepared by adding 12 µL of Pro260 gel and gel stain to the designated wells. The chip was then placed on the priming station and primed for 1 min at the medium (B) pressure setting. The sample was reduced with dithiothreitol (Sigma-Aldrich) and denatured in the kit-provided sample buffer at 95 °C before it was applied to the primed chip. The instrument was operated using the Experion software. All samples were run in triplicate alongside one sample of the control (BGG) and the Pro260 ladder. Analysis was performed using the Experion software.

2.2.3 Protein Behavior Measurement

Dynamic light scattering (DLS) was used to evaluate how the proteins behaved in solution. Three tests are performed to determine protein uniformity in solution. Polydispersity is a measure of the size distribution of particles in solution. For protein molecules, a polydispersion value of less than 20% is considered monodisperse. The hydrodynamic radius and molecular weight of the sample are displayed by two graphs. The correlation graph indicates the relative particle size, and the steepness of the line indicates the monodispersion level of the sample. The regularization graph (derived from the data) shows the hydrodynamic radius, percent mass, and molecular weight. The DLS software uses prediction algorithms to produce this range of values for the protein under evaluation.

For DLS analysis, five 20 µL aliquots of the antibody, along with the control bovine serum albumin (Sigma-Aldrich), were placed into a quartz 384-well plate (Wyatt Technology Corp.; Santa Barbara, CA) and centrifuged (2 min, 239×*g*) to remove trapped air bubbles from the samples. Mineral oil (Sigma-Aldrich) was applied to the top of each sample to prevent sample evaporation, and the plate was then placed into a temperature-controlled DynaPro plate reader (Wyatt Technology). Each well was scanned 10 times for 5 s each at 25 °C. Results were averaged, and the Wyatt Technology Dynamics software was used to measure polydispersity, hydrodynamic radius, percent mass, and molecular weight for each sample. The results for three wells were averaged and reported.

2.2.4 Thermal Stability Measurement

Differential scanning calorimetry (DSC) was used to obtain a quantitative melting temperature (T_m) for each of the antibody proteins. The T_m is determined to predict results of subsequent enzyme-linked immunosorbent assay (ELISA) and surface plasmon resonance (SPR) thermostability testing. A T_m above 70 °C predicts that antibody activity after the thermal stress test will remain above 50%. A T_m below 70 °C predicts, at minimum, a 50% decrease in

antibody activity after the thermal stress test. For DSC experiments, samples were diluted to 0.5 mg/mL and dialyzed overnight in PBS at pH 7.4. Samples were degassed for 5 min before analysis and injected into the sample cell of a VP-DSC calorimeter (MicroCal; Northampton, MA). Dialysis buffer was added to the reference cell of the calorimeter, and a buffer scan was used as the baseline for all experiments. The samples (in duplicate) were scanned from 15 to 100 °C at a rate of 60 °C/h. The transition midpoint T_m of the protein was determined by data analysis with Origin 7.0 software (MicroCal).

2.2.5 Thermal Stress Testing

All samples were diluted to a concentration of 1 mg/mL before heat was applied to negate protective effects due to concentration. Antibodies were diluted to 1 mg/mL in 1× PBS and divided into five tubes. One aliquot was kept on ice for the duration of the experiment and was marked time 0. The remaining four aliquots were heated to 75 °C on a calibrated heat block for 15, 30, 45, and 60 min each. After each time point, the corresponding aliquot was removed and placed in an ice bath. These samples were then tested for activity using ELISA.

2.2.6 ELISA Methodology

After thermal testing was complete, ELISAs were performed (in triplicate) using standard capture assay techniques. Each antibody sample was diluted to 20 µg/mL in PBS. A twofold serial dilution was performed across each Nunc MaxiSorp 96-well plate (Thermo Fisher Scientific). Samples were incubated at 4 °C overnight. In the morning, each plate was washed in 1× wash buffer (KPL/SeraCare; Gaithersburg, MD) using a standard wash protocol on an AquaMax 200 plate washer (MDS Analytical Technologies; Sunnyvale, CA) and then blocked with KPL 1× milk diluent block (MDB) for 30 min at 37 °C. The plates were then washed again, and 100 µL of 0.125 µg/mL biotinylated Bc1A antigen was added to each well. The plates were incubated for 1 h at room temperature. Horseradish peroxidase (HRP)-labeled streptavidin (KPL/SeraCare) was diluted to 0.1 µg/mL in 1× MDB, 100 µL of which was added to each well and incubated at room temperature for 1 h. After plates were washed, 100 µL of room-temperature ABTS 1-component HRP substrate (KPL/SeraCare) was added to each well. After 9 min at room temperature, the optical density at the 405 nm light wavelength was determined using a Synergy H4 hybrid multimode microplate reader (BioTek; Winooski, VT). Data analysis was performed using Prism software, version 5.00 for Windows (GraphPad Software; San Diego, CA).

2.2.7 SPR Methodology

SPR is a method used to determine the kinetic parameters of an antibody–antigen interaction. It is a rapid means for monitoring biomolecular interactions through the excitation of surface plasmons, which results when polarized light is shone through a prism onto a sensor chip with a thin metal film coating. The metal film acts as a mirror and reflects the light. When the angle of light shining through the prism is changed, the intensity of the reflected light also changes. These intensity differences can be monitored and recorded. Although the refractive index at the prism side of the chip does not change, the refractive index in the immediate vicinity of the metal surface does change when accumulated mass (bound proteins) adsorbs onto the surface. Therefore, if binding occurs, the resonance angle (SPR angle) changes, and this shift of

the SPR angle provides information about the kinetics of the protein adsorption on the surface. The SPR software can then be used to accurately analyze the association and dissociation rate constants (k_a and k_d, respectively) for the antibody interactions and to calculate the overall equilibrium dissociation constants (K_D values) between antibodies and antigens.

2.2.7.1 Thermostability Testing

On a Biacore T200 system (GE Healthcare Life Sciences; Marlborough, MA), 6500 response units (RU) of Bc1A were tethered to one flow cell of a Biacore CM5 chip using standard amine coupling chemistry. After thermal stress testing was performed, samples were centrifuged at 5 °C and 2000×*g* for 5 min. The analyte was run at 10 µL/min for 120 s. A calibration curve was created by injecting eight concentrations of the time 0 unheated antibody at 400, 350, 300, 250, 200, 150, 100, and 50 nM and then plotting the respective maximum analyte-binding capacities of the surface (R_{Max}) in response units. Unheated and heated samples were diluted 1:90 and 1:180 so that the time 0 control points would fall on the linear calibration curve. All samples were run in triplicate. The chip surface was regenerated with an 18 s injection of 0.85% phosphoric acid at a flow rate of 30 µL/min. Data were collected using Biacore T200 Evaluation concentration analysis software (GE Healthcare Life Sciences), and the active concentrations of heated samples were recorded. The running buffer used for this experiment was Biacore 1× HBS-EP buffer (GE Healthcare Life Sciences).

2.2.7.2 Kinetic Analysis

Using the Biacore T200 system and 1× HBS-EP running buffer, 102 RU of Bc1A was tethered to a Biacore CM5 chip using standard amine coupling chemistry. MBP-sdAb was injected across the chip surface for 120 s at a flow rate of 75 µL/min with a 900 s dissociation at 60, 20, 6.67, and 2.2 nM and 700 pM. The chip surface was regenerated using an 18 s injection of 0.85% phosphoric acid at 30 µL/min with a 60 s stabilization period. Data were analyzed using a Langmuir 1:1 fit.

3. RESULTS

3.1 Antibody Concentration Measurements

3.1.1 MBP-sdAb Results

A_{280} values for MBP-sdAb were obtained in triplicate on the NanoDrop ND-1000 spectrophotometer. The A_{280} readings are listed in Table 1.

Table 1. A_{280} Readings for MBP-sdAb

Replicate No.	A_{280} Value
1	3.821
2	3.821
3	3.794

An average was determined from the A_{280} results, and it was divided by the extinction coefficient of 1.6. The reported concentration was 2.38 mg/mL.

3.1.2 BA21 Results

A_{280} values for BA21 were obtained in triplicate on the NanoDrop ND-1000 spectrophotometer. The A_{280} readings are listed in Table 2.

Table 2. A_{280} Readings for BA21

Replicate No.	A_{280} Value
1	1.19
2	1.19
3	1.18

An average was determined from the A_{280} results, and the spectrophotometer software automatically divided it by the extinction coefficient. The reported concentration was 1.2 mg/mL.

3.2 Molecular Weight and Purity Measurements

3.2.1 MBP-sdAb Molecular Weight and Purity

The molecular weight of the MBP-sdAb was determined (Figure 1) using the Experion Pro260 Analysis Kit. In the figure, the thick band at the top of the second lane corresponds to the antibody single chain. According to the software, the antibody was 85.4% pure, and the chain weighed 69.5 kDa.

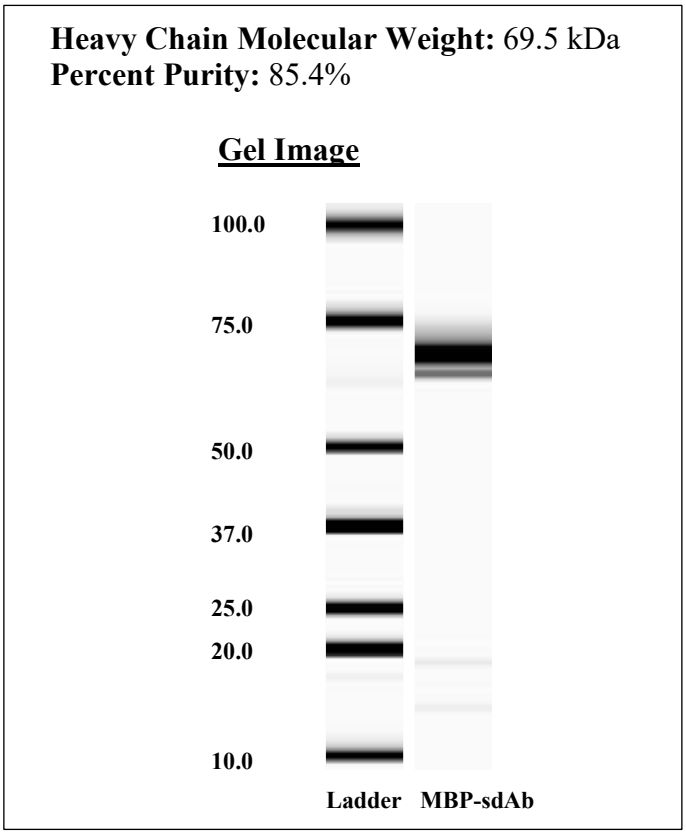


Figure 1. Molecular weight and purity of MBP-sdAb. Digital gel of MBP-sdAb produced by the Experion Pro260 system. The thick band at the top of the right lane corresponds to the MBP-sdAb single chain.

3.2.2 BA21 Molecular Weight and Purity

The molecular weight of BA21 was measured (Figure 2) using the Experion Pro260 Analysis Kit. In the figure, the thick band at the top of the second lane corresponds to the antibody heavy chain, and the lighter band corresponds to the light chain. According to the software, the antibody was 98.4% pure, the heavy chain weighed 70.0 kDa, and the light chain weighed 27.5 kDa.

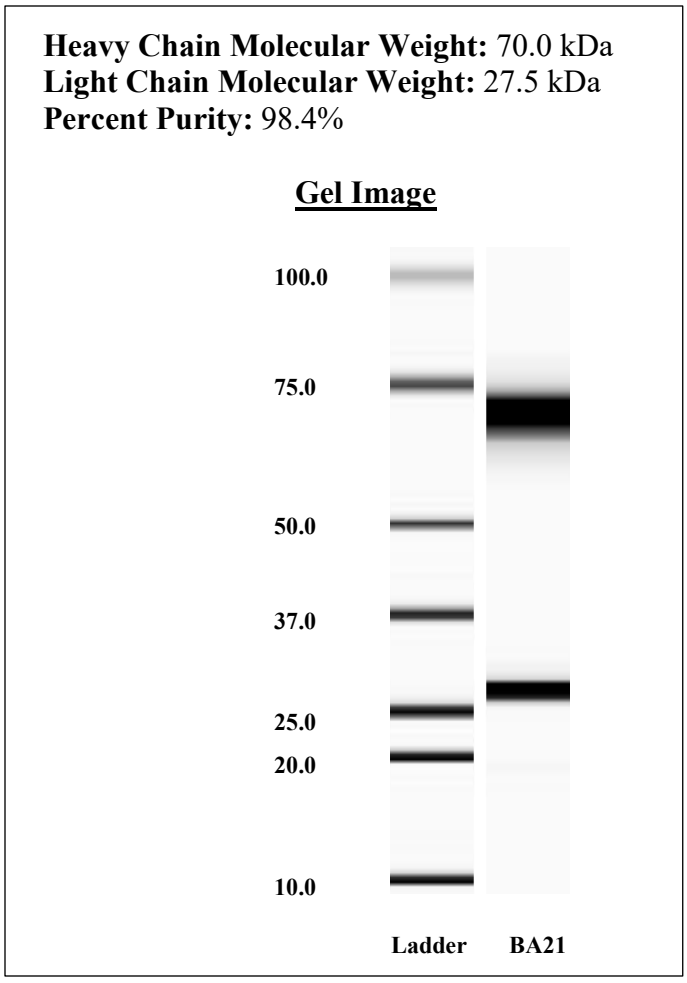


Figure 2. Molecular weight and purity of BA21. Digital gel of BA21 produced by the Experion Pro260 system. The thick band at the top of the right lane corresponds to the BA21 heavy chain, and the thinner band at the bottom of the second lane corresponds to the light chain.

3.3 Protein Behavior by DLS

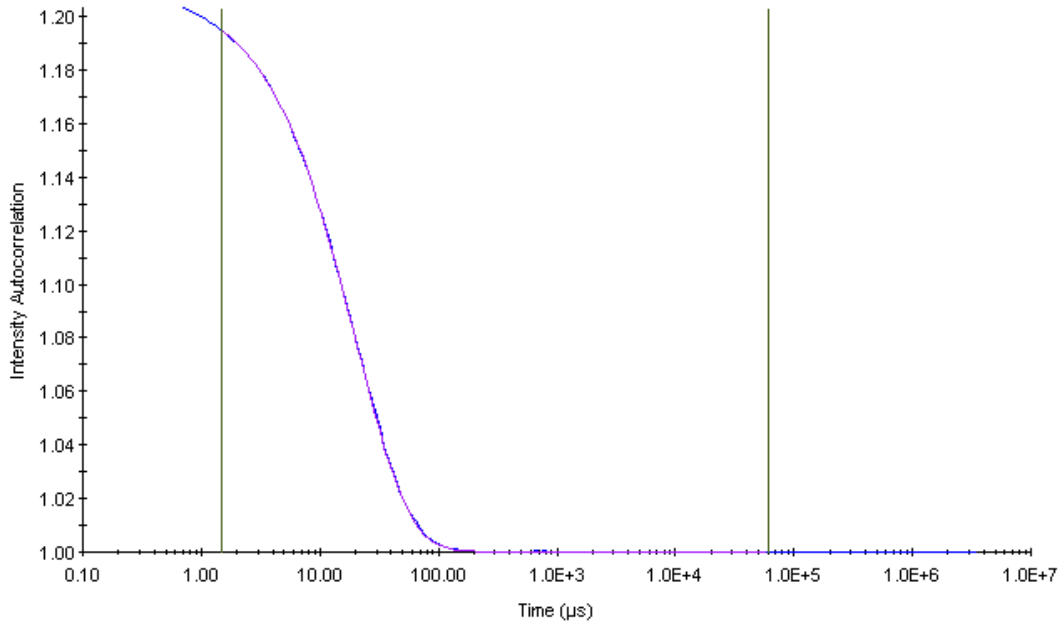
3.3.1 MBP-sdAb Protein Behavior

The MBP-sdAb was analyzed in triplicate using the DynaPro plate reader. The radius of MBP-sdAb was determined to be 3.8 nm, and the polydispersity was 9.4% (Table 3). Figure 3 shows representative correlation and regularization graphs for the MBP-sdAb. The correlation graph (panel A) depicts a sigmoidal curve indicative of a valid size distribution. The regularization graph (panel B) illustrates the monodispersity of the sample. Table 3 lists the raw data that were produced for each replicate. Because 100% of the mass displayed favorable polydispersity and hydrodynamic radius, both of these sample preparations were considered to be monodisperse.

Table 3. Features of MBP-sdAb in Solution

Replicate No.	Radius (nm)	Polydispersity (%)
1	3.6	8.2
2	4.0	16.1
3	3.7	7.5
4	3.8	6.1
5	3.8	9.2
Average	3.8	9.4

A. Correlation Graph for MBP-sdAb



B. Regularization Graph for MBP-sdAb

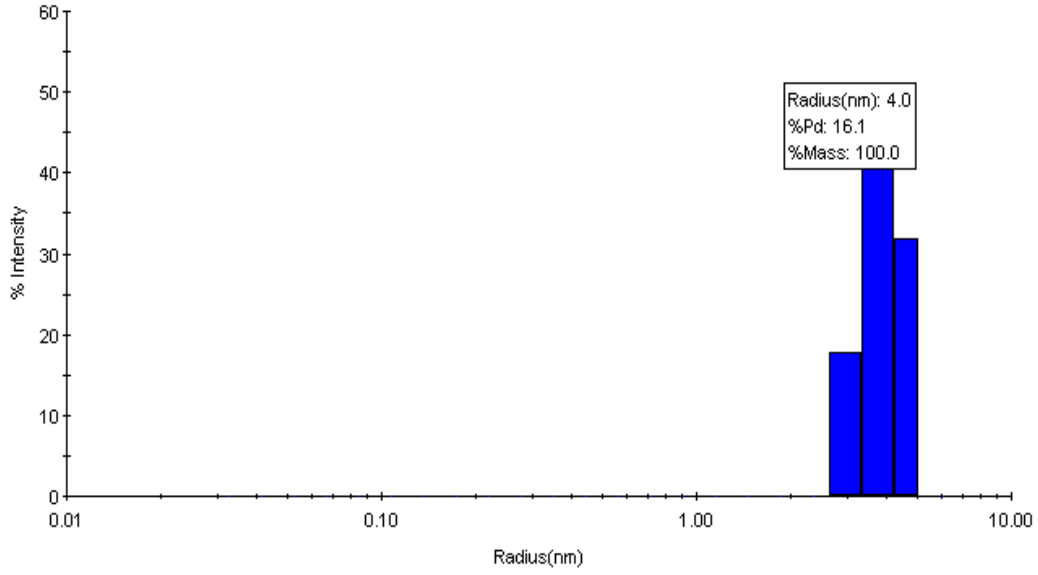


Figure 3. Radius and polydispersity representation for MBP-sdAb:
(A) correlation graph and (B) regularization graph.

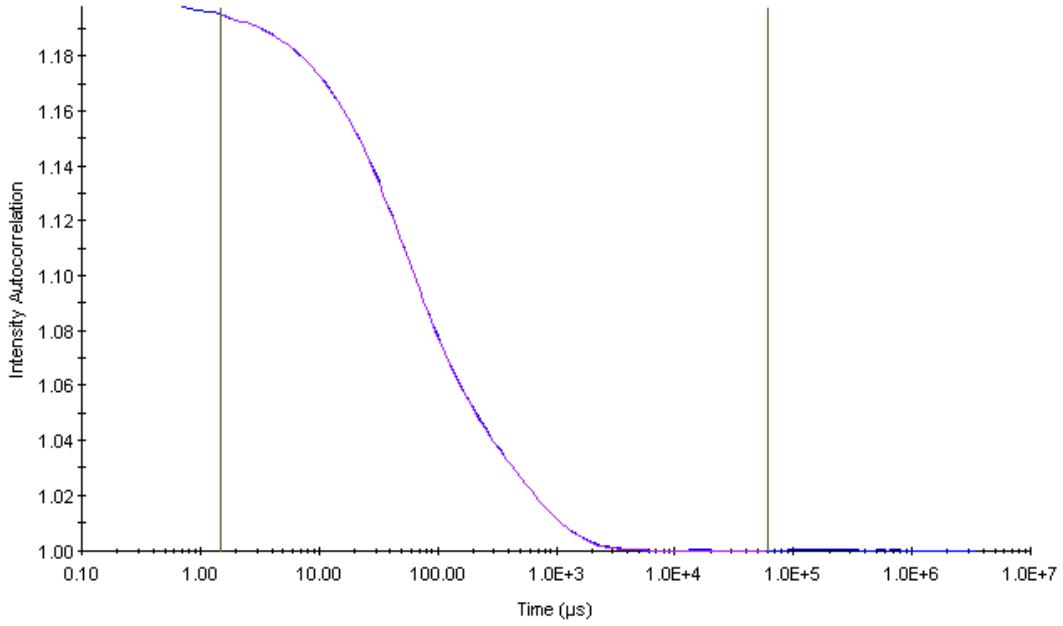
3.3.2 BA21 Protein Behavior

DLS analysis was performed of BA21 in solution. The radius of BA21 was determined to be 7.3 nm, and the polydispersity was 38.3% (Table 4). Figure 4 contains representative correlation and regularization graphs for BA21. The correlation graph (panel A) depicts a sigmoidal curve indicative of a valid size distribution. The regularization graph (panel B) illustrates the monodispersity identified in both samples. Table 4 lists the raw data produced for each replicate. Because 100% of the mass displayed favorable polydispersity and hydrodynamic radius, the sample preparation was considered to be monodisperse.

Table 4. Features of BA21 in Solution

Replicate No.	Radius (nm)	Polydispersity (%)
1	8.1	50.4
2	7.1	36.8
3	7.6	43.0
4	6.3	22.9
Average	7.3	38.3

A. Correlation Graph for BA21



B. Regularization Graph for BA21

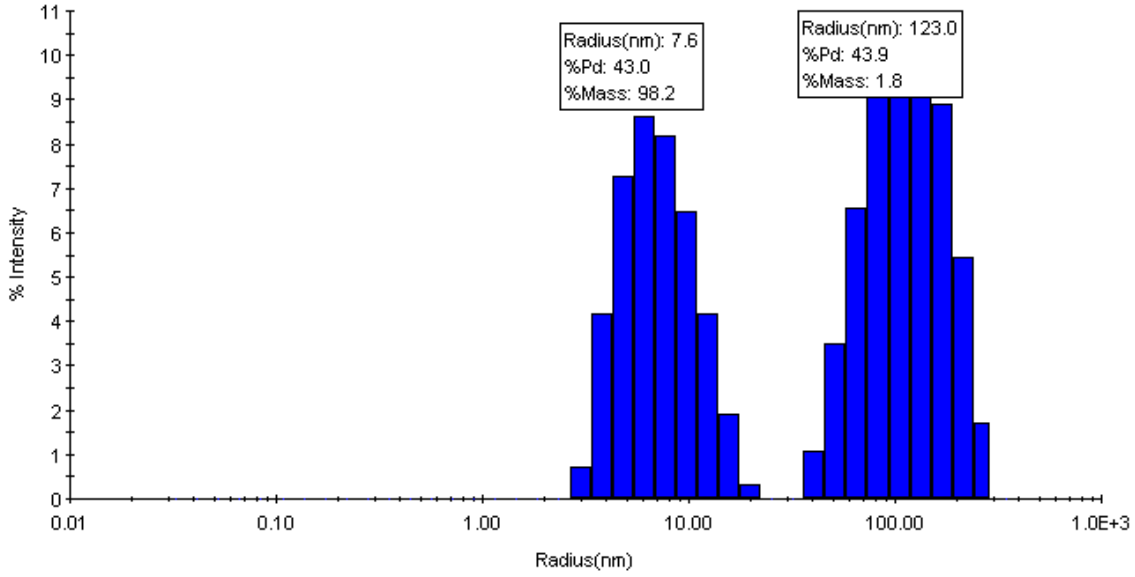


Figure 4. Radius and polydispersity representation for BA21:
(A) correlation graph and (B) regularization graph.

3.4 DSC

3.4.1 MBP-sdAb Melting Temperature

Readings for MBP-sdAb were obtained in duplicate on the MicroCal VP-DSC calorimeter. The peak melting temperatures are shown in Figure 5.

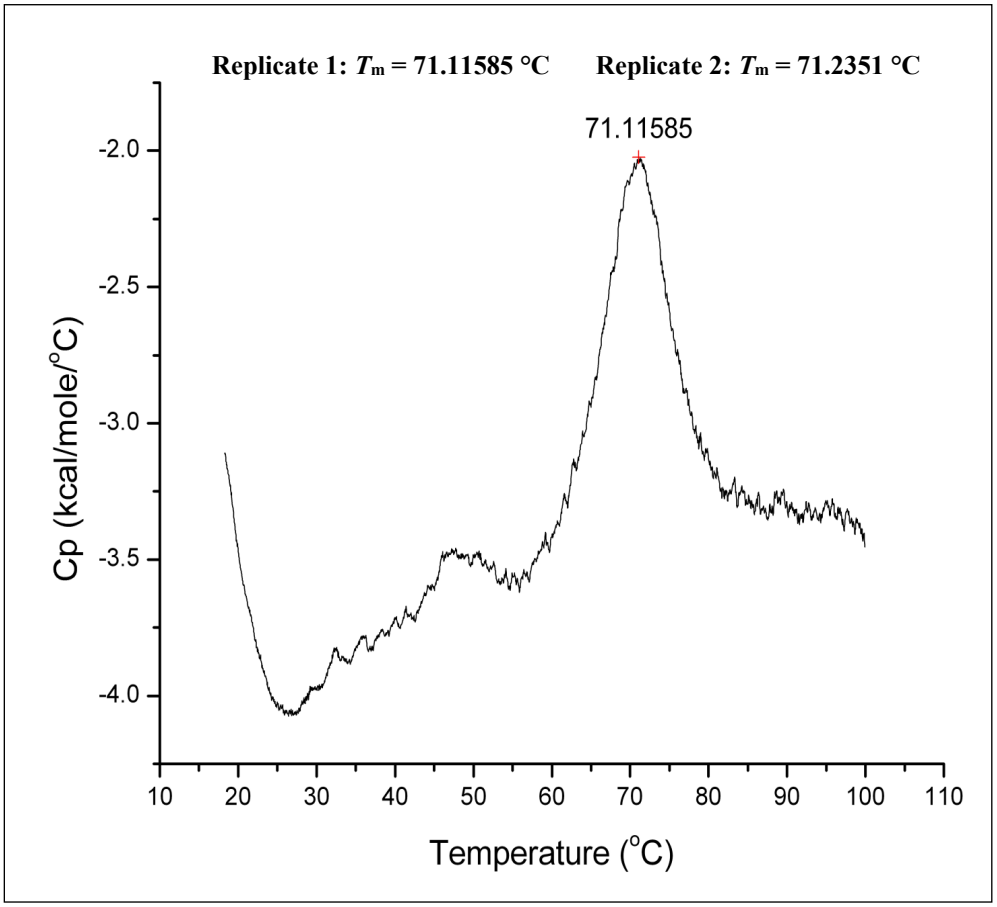


Figure 5. Transition midpoint curve for MBP-sdAb. T_m was calculated to be 71.1 °C.

3.4.2 BA21 Melting Temperature

Readings for BA21 were obtained on the MicroCal VP-DSC calorimeter. The peak melting temperature is shown in Figure 6.

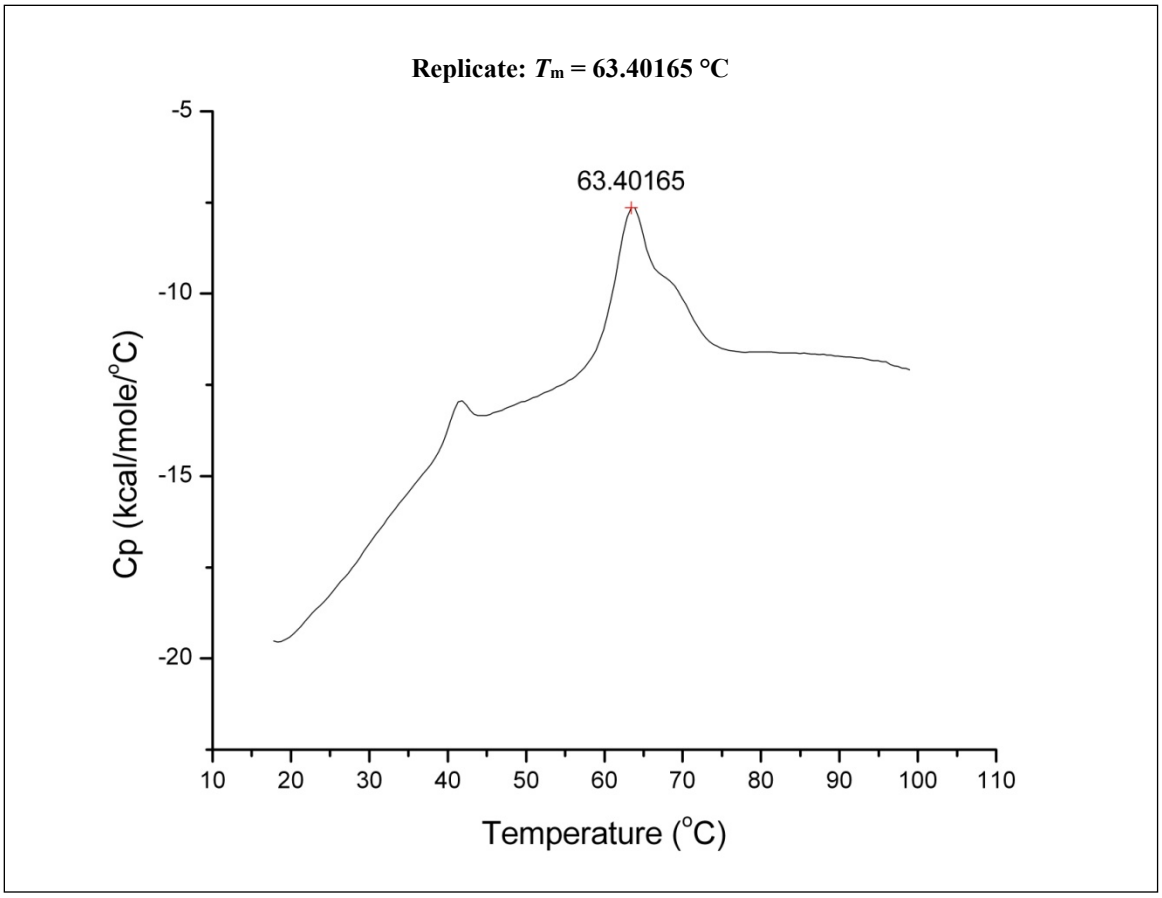


Figure 6. Transition midpoint curve for BA21. T_m was calculated to be 63.4 °C.

3.5 Post-Temperature-Stress ELISA

3.5.1 MBP-sdAb ELISA

ELISAs were used to test the functional interactions of antibodies and antigens after thermal stress at 70 °C. The area under the curve for each of the different time points at 70 °C was calculated, averaged, and graphed to depict how the MBP-sdAb reacted to thermal stress over time. Figure 7 illustrates that the antibody remained functionally capable of binding to antigen after a 60 min exposure to 70 °C.

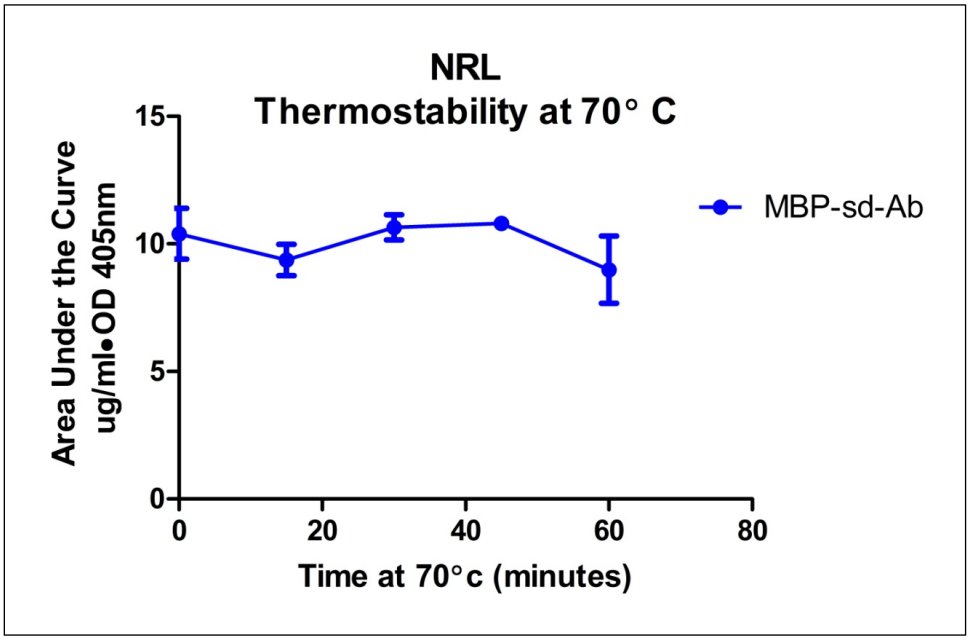


Figure 7. Thermostability of MBP-sdAb ELISA. Area under the curve analysis depicts the effect of thermal stress.

3.5.2 BA21 ELISA

ELISAs were used to test the functional interactions of BA21 under thermal stress conditions. The ELISA data shown in Figure 8 indicate that when the BA21 was heated to 70 °C, it lost all activity after about 15 min.

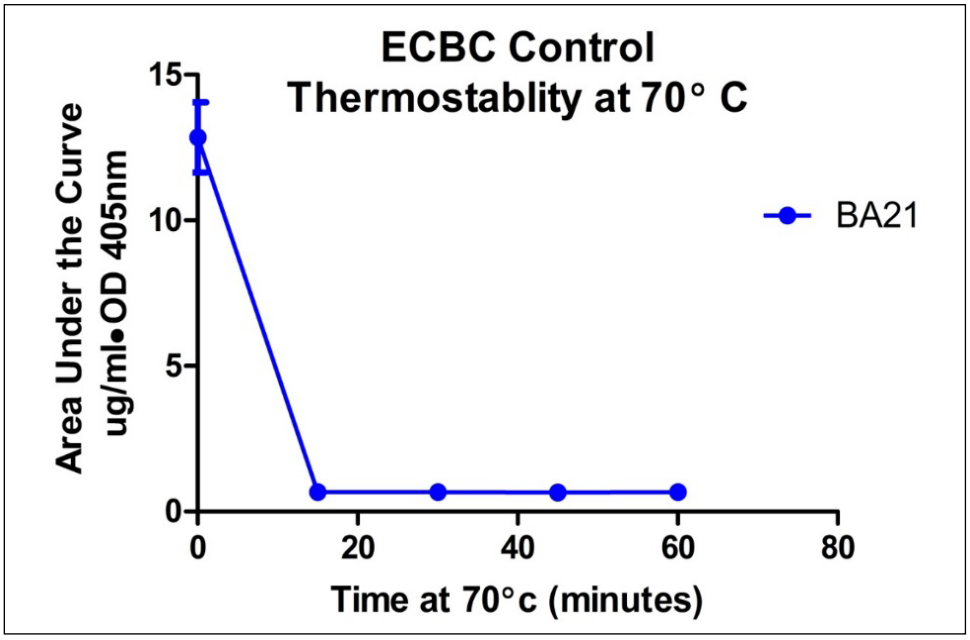


Figure 8. Thermostability of BA21 ELISA. Area under the curve analysis depicts the effect of thermal stress.

3.6 SPR

3.6.1 Thermostability Testing by SPR

SPR was used to assess the functional bindings between MBP-sdAb and antigens after heating to 70 °C for various time periods. Five tubes of 1 mg/mL were prepared and heated to 70 °C for 15, 30, 45, and 60 min time periods and were then quenched on ice. The Biacore T200 system was used to compare the activity of each sample to a calibration curve constructed from unheated sample data (Figures 9 and 10). This activity curve (Figure 9) showed that the NRL MBP-sdAb retained over 90% of its activity at 70 °C for 60 min. This was a greater resistance to heat than was exhibited by BA21 (Figure 10), which lost all activity after 15 min at 70 °C.

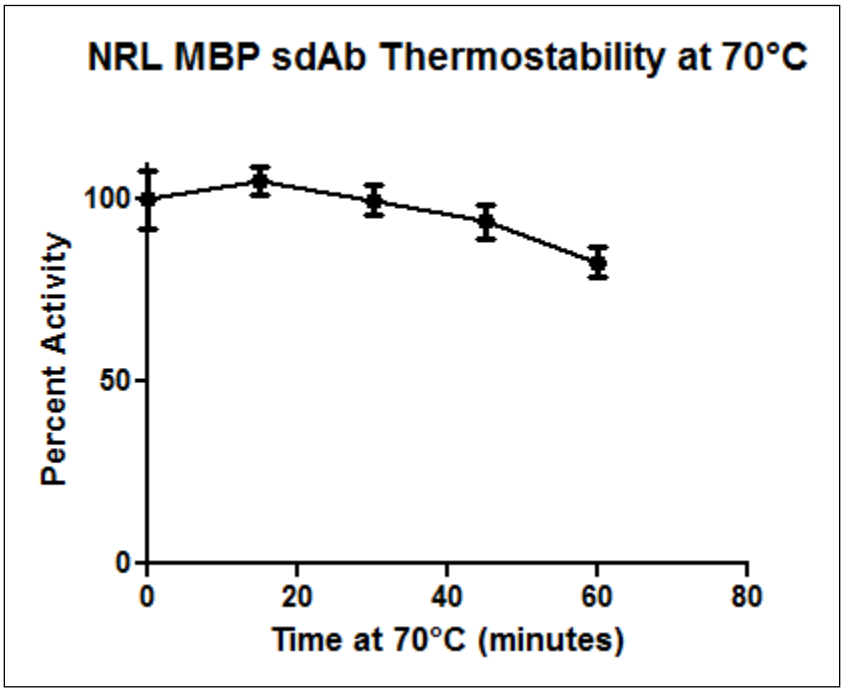


Figure 9. Thermostability of MBP-sdAb as assessed using SPR. MBP-sdAb maintained over 90% of its activity after it was heated to 70 °C for 60 min.

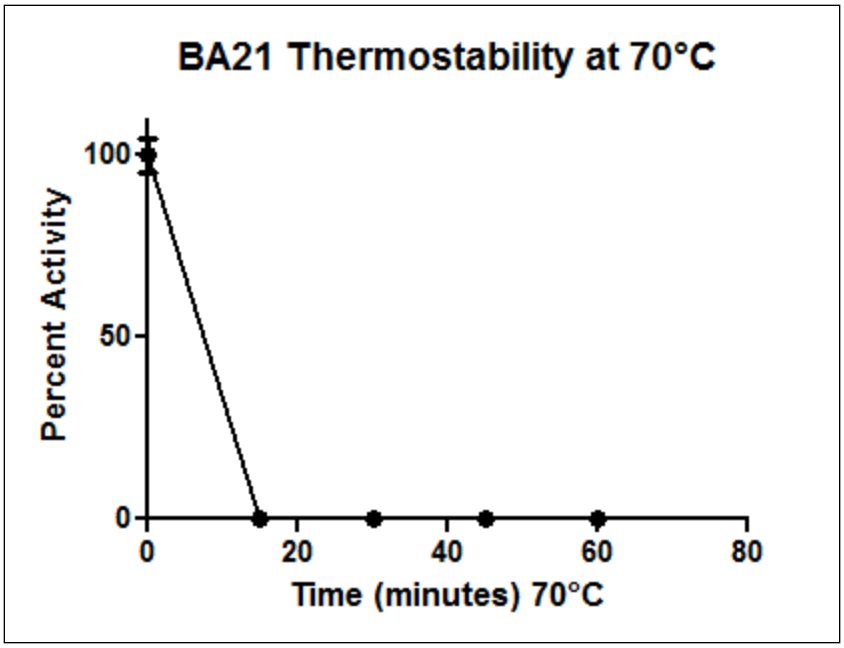


Figure 10. Thermostability of the BA21 as assessed using SPR. When BA21 was heated to 70 °C, it lost all activity after 15 min.

3.6.2 Kinetic Analysis by SPR

Kinetic analysis of the NRL MBP-sdAb binding to the BA21 antigen was performed as a direct-binding SPR experiment on the Biacore T200 system. Results are presented in Figure 11. Data were normalized to a blank-immobilized reference flow cell and then fit to a Langmuir 1:1 model using Biacore T200 software. The K_D was determined to be 5.3 pM. Data from similar experiments that were performed using the original BA21 are presented in Figure 12. The K_D was determined to be 4.99 nM; therefore, NRL provided an antibody that was well above the 100-fold improvement threshold.

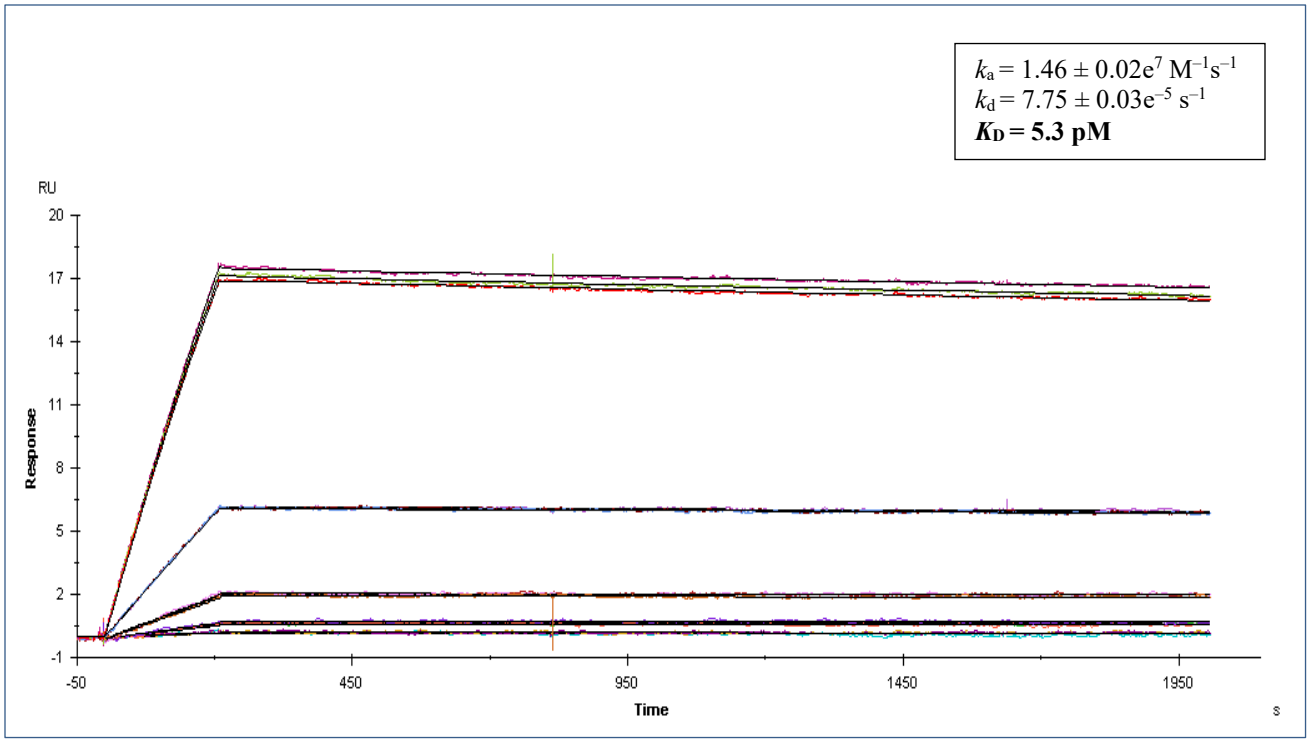


Figure 11. Kinetics of MBP-sdAb as determined using a Biacore T200 system.

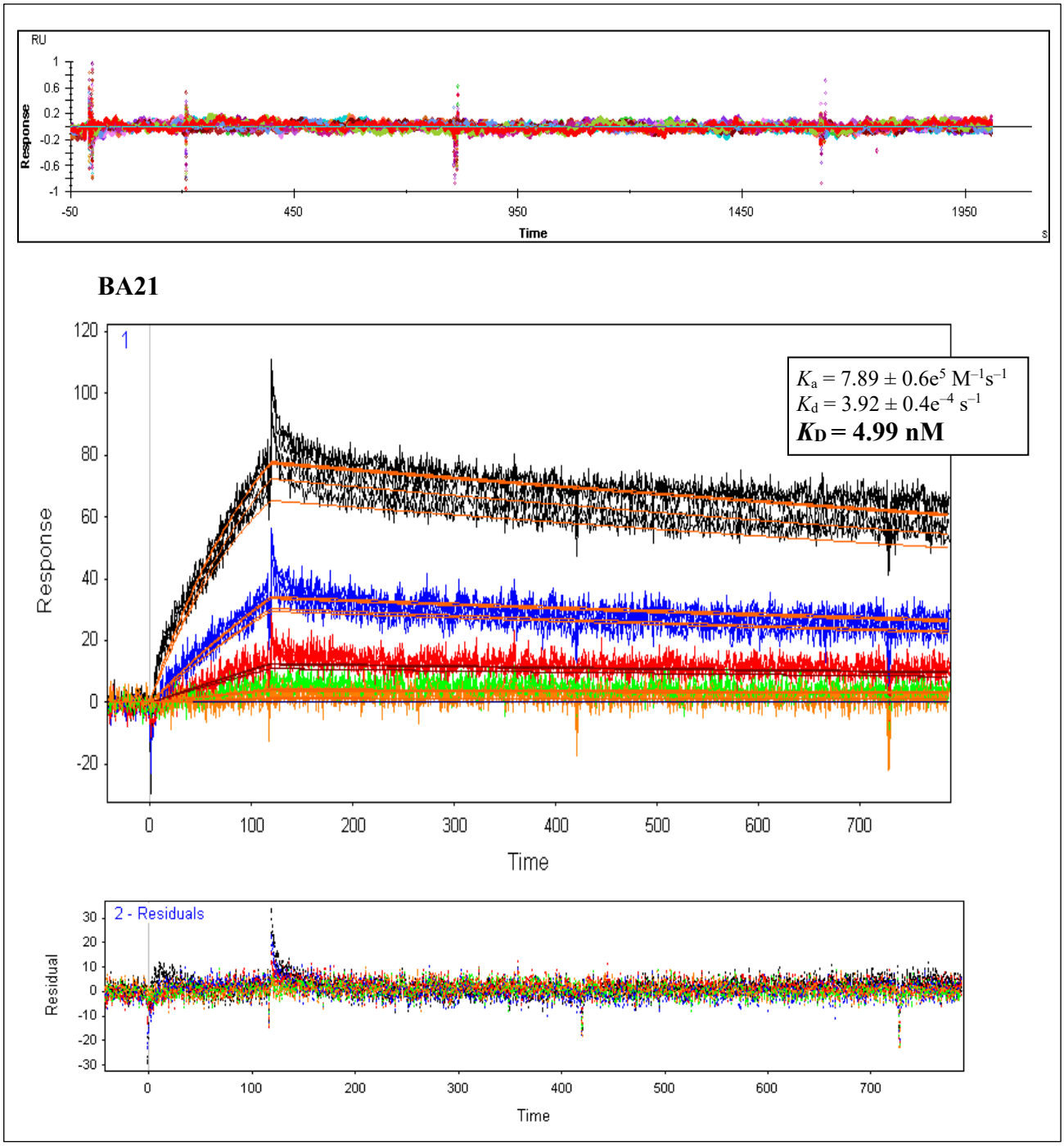


Figure 12. Kinetics of BA21 as determined using a Biacore T200 system.

4. DISCUSSION

In this study, standardized parametric tests were performed that were established during the MS2 scFv antibody DARPA ATP. The performer was able to demonstrate their molecular schemes for improving the thermal stability and affinity of an antibody for its target antigen. Images of the BA21 physical characteristics were obtained using the NanoDrop ND-1000, Experion, and DLS measurement platforms. These characteristics were compared with those of the improved antibodies submitted by NRL. Measurements of the MBP-sdAb functional characteristics were obtained using the DSC, ELISA, and SPR analytic platforms. These measurements were used to assess the effects of molecular engineering on thermal stability and affinity.

An accurate assessment of protein concentration was critically important for all of the test procedures described in this report. We applied a standard spectrophotometry technique using a NanoDrop ND-1000 system. With this instrument, we obtained A_{280} values of the samples. A_{280} values are influenced by the number of tryptophan and tyrosine residues in a given protein. For this reason, extinction coefficients were used in conjunction with A_{280} readings to determine accurate concentrations.

After concentrations were determined with the NanoDrop ND-1000 spectrophotometer, molecular weight and purity data were collected with the Experion automated electrophoresis system. This system uses microfluidic technology to automate electrophoresis for protein analysis. The results of Experion analysis of the NRL antibody fell within the acceptable purity range for use in assay development, and the molecular weight was determined by the software.

DLS was used in conjunction with the Experion and NanoDrop ND-1000 systems to evaluate how the proteins behaved in solution. DLS data indicated the physical state and potential aggregation of a protein in solution by measuring the polydispersity, hydrodynamic radius, and molecular weight of a sample. The DLS data established whether the MBP-sdAb provided by NRL was monomeric and monodisperse. Less than 1% of the sample mass appeared to be aggregating in solution (Figure 2). To mitigate the exacerbating effect of freeze–thawing on future sample aggregation and to ensure that all testing would be consistent, the NRL MBP-sdAb samples were aliquoted into single-use vials and centrifuged before use.

SPR was also used to obtain a kinetic analysis of the enhanced NRL MBP-sdAb binding to its target antigen BA21, to compare binding parameters with those for the original antibody. The K_D values clearly showed that NRL well-exceeded the 100-fold improvement that was requested by DARPA.

5. CONCLUSION

The DARPA ATP sought to establish methods for rapidly engineering a given antibody reagent to exhibit physical and functional properties that far exceeded those of its native state. This is necessary to expand user confidence in fielding antibody-based detection and diagnostic platforms in environments and operational scenarios that degrade or interfere with the

currently available reagents. By optimizing the thermal stability and binding affinity of an antibody for its biological target, the DARPA ATP sought to yield antibody reagents that can reliably function in harsh environmental conditions, and thereby increase the sensitivity of a sensor platform to detect lower levels of a threat agent.

This report documents the testing of an improved antibody produced by NRL. The physical and functional characteristics of an antibody in the ECBC testing pipeline were evaluated. The results were compared with baseline characteristics of the original antibody's physical properties, to include concentration, molecular weight, purity, and state of aggregation in solution, as well as functional measures such as binding affinity and thermal stability. The antibody supplied by NRL exhibited enhanced thermal stability and affinity for binding to the BA21 protein antigen.

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ACRONYMS AND ABBREVIATIONS

A_{280}	absorbance of light at 280 nm
ATP	Antibody Technology Program
BGG	bovine γ-globulin
DARPA	Defense Advanced Research Projects Agency
DLS	dynamic light scattering
DSC	differential scanning calorimetry
ECBC	U.S. Army Edgewood Chemical Biological Center
ELISA	enzyme-linked immunosorbent assay
HRP	horseradish peroxidase
k_a	association rate constant
k_d	dissociation rate constant
K_D	equilibrium dissociation constant
mAb	monoclonal antibody
MDB	milk diluent block
NRL	U.S. Naval Research Laboratory
PBS	phosphate-buffered saline
R_{Max}	maximum analyte-binding capacity of the surface
RU	response unit
scFv	single-chain variable fragment
sdAb	single-domain antibody
SPR	surface plasmon resonance
T_m	melting temperature

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